

AMENDMENTS TO THE CLAIMS

The listing of claims provided below will replace all prior versions, and listings, of claims in the application.

Listing of Claims

1-41. (Canceled)

42. (Currently Amended) An isolated or purified nucleic acid encoding a polypeptide having comprising the amino acid sequence of SEQ ID NO:4, ~~said amino acid sequence comprising at least one immunogenic epitope.~~

2 43. (Currently Amended) The nucleic acid of claim 42 or 87, wherein ~~said~~ the nucleic acid comprises nucleotides 13-1314 of SEQ ID NO:3.

44-47 (Canceled)

8 48. (Currently amended) A method of preparing a polypeptide comprising a carboxy-terminal portion of the heavy chain of botulinum neurotoxin serotype A ~~comprising at least one immunogenic epitope~~, comprising:

transfecting a host cell with a nucleic acid encoding a polypeptide having the amino acid sequence of SEQ ID NO:4, ~~said amino acid sequence comprising at least one immunogenic epitope~~; and

culturing the transfected host cell under conditions wherein the nucleic acid is expressed and wherein the polypeptide is produced from the nucleic acid,

wherein the host cell is selected from the group consisting of a gram negative bacteria, a yeast, and a mammalian cell.

49-52. (Canceled)

|| ~~53.~~ (Currently amended) A method of isolating an immunogenic polypeptide ~~having~~
comprising the amino acid sequence of SEQ ID NO:4, ~~said amino acid sequence comprising~~
~~at least one immunogenic epitope~~, comprising:

culturing a host cell transfected with an expression vector comprising a nucleic acid
encoding a polypeptide ~~having~~ comprising the amino acid sequence of SEQ ID NO:4, ~~said~~
~~amino acid sequence comprising at least one immunogenic epitope~~, under conditions
wherein the nucleic acid is expressed and wherein the polypeptide is produced from the
nucleic acid; and

isolating from ~~said~~ the transfected host cell at least one polypeptide comprising the
amino acid sequence of SEQ ID NO:4, ~~said amino acid sequence comprising at least one~~
~~immunogenic epitope~~,

wherein the host cell is selected from the group consisting of a gram negative
bacteria, a yeast, and a mammalian cell, and wherein the recovered polypeptide is
immunogenic.

54. (Canceled)

3 ~~55.~~ (Currently Amended) The nucleic acid of claim ~~42~~ ¹ or ~~87~~, wherein ~~said~~ the nucleic acid
has an overall AT content of less than 70% of the total base composition.

4 ~~56.~~ (Currently Amended) The nucleic acid of claim ~~55~~ ³, wherein ~~said~~ the nucleic acid has an
overall AT content of less than about 60% of the total base composition.

57-81. (Canceled)

5 ~~82.~~ (Previously Presented) A recombinant host cell comprising the nucleic acid of claim

~~42.~~

83-84. (Canceled)

6 ~~85.~~ (Currently Amended) The recombinant host cell of claim ~~82~~⁵, wherein the host cell expresses the nucleic acid and wherein a polypeptide having the amino acid sequence of SEQ ID NO:4 is expressed, wherein ~~said~~ the polypeptide is at least 0.75% (w/w) of the total cellular protein.

7 ~~86.~~ (Currently Amended) The recombinant host cell of claim ~~85~~⁶, wherein ~~said~~ the polypeptide is at least 20% (w/w) of the total cellular protein.

14 ~~87.~~ (Previously Presented) An isolated or purified nucleic acid comprising the nucleic acid sequence of SEQ ID NO:3.

9 ~~88.~~ (Previously Presented) A method of preparing a polypeptide comprising a carboxy-terminal portion of the heavy chain of botulinum neurotoxin serotype A, comprising:

transfecting a host cell with a nucleic acid having the nucleic acid sequence of SEQ ID NO:3,

culturing the transfected host cell under conditions wherein the nucleic acid is expressed and wherein a polypeptide is produced from the nucleic acid,

wherein the host cell is selected from the group consisting of a gram negative bacteria, a yeast, and a mammalian cell.

10 ~~89~~ (Currently Amended) The method of claim ~~48~~ or ~~88~~, further comprising recovering
from the transfected host cell at least one polypeptide having the amino acid sequence of
SEQ ID NO:4, wherein ~~said~~ the polypeptide is an insoluble polypeptide.

90. (Canceled)

12 ~~91~~ (Currently Amended) A method of isolating an immunogenic polypeptide having the
amino acid sequence of SEQ ID NO:4, comprising:

culturing a host cell transfected with an expression vector comprising ^{the} ~~a~~ nucleic acid
having the sequence of SEQ ID NO:3 under conditions wherein the nucleic acid is expressed
and wherein the polypeptide is produced from the nucleic acid; and

isolating from ~~said~~ the transfected host cell at least one polypeptide comprising the
amino acid sequence of SEQ ID NO:4,

wherein ~~said~~ the host cell is selected from the group consisting of a gram negative
bacteria, a yeast and a mammalian cell, and wherein ~~said~~ the isolated polypeptide is
immunogenic.

13 ~~92~~ (Currently Amended) The method of claim ~~53~~ or ~~91~~, wherein ~~said~~ the polypeptide is
an insoluble polypeptide.

~~Canceled~~ (Newly Added) An isolated or purified nucleic acid comprising the nucleotides 13-
1314 of SEQ ID NO:3.

15 ~~94~~ (Newly Added) The nucleic acid of claim ~~42~~ or ~~87~~, wherein the nucleic acid is an
isolated nucleic acid.

16 ~~95~~. (Newly Added) The nucleic acid of claim ~~42~~¹ or ~~87~~¹⁴, further comprising an expression control sequence operably linked to the nucleotide sequence.

17 ~~96~~. (Newly Added) The nucleic acid of claim ~~95~~¹⁶, wherein the expression control sequence comprises a promoter.

18 ~~97~~. (Newly Added) The nucleic acid of claim ~~95~~¹⁶, wherein the expression control sequence comprises an enhancer.

19 ~~98~~. (Newly Added) The method of claim ~~48~~⁸ or ~~88~~⁹, further comprising recovering from the transfected host cell at least one polypeptide having the amino acid sequence of SEQ ID NO:4.

20 ~~99~~. (Newly Added) The method of claim ~~98~~¹⁹, wherein the recovered polypeptide is an insoluble polypeptide.

21 ~~100~~. (Newly Added) The method of claim ~~48~~⁸ or ~~88~~⁹, wherein the host cell is *Escherichia coli*.

22 ~~101~~. (Newly Added) The method of claim ~~48~~⁸ or ~~88~~⁹, wherein the host cell is *Pichia pastoris*.